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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/262,126	03/03/1999	BRIAN S. MILLER	GC396-2	8961

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GENENCOR INTERNATIONAL, INC.
ATTENTION: LEGAL DEPARTMENT
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EXAMINER	
RAO, MANJUNATH N	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 05/04/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/262,126	MILLER ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 February 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *5-10,12,14,15,27-40 and 52-66* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5-7,9,10,12,14,15,27-40,52,53 and 55-66* is/are rejected.

7)☒ Claim(s) *8,30 and 54* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Request for Continued Examination

The request filed on 2-13-04 for a Continued Examination (RCE) under 37 CFR 1.114 based on parent Application No. 09/262,126 is acceptable and a RCE has been established. An action on the RCE follows.

Claims 5-10, 12, 14-15, 27-40, 52-66 are currently pending in this application.

Applicants' amendments and arguments filed on 2-13-04, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. However, after perusal of the references used in the previous rejections and applicant's arguments in response to the previous rejection, Examiner has re-instituted the obviousness rejection.

Sequence Compliance

Applicant is required to comply with the sequence rules by inserting the sequence identification numbers of all sequences recited within the claims and/or specification. It is particularly noted that claims 60-64 recite the amino acid sequence VWAP without providing any SEQ ID NO:. See particularly 37 CFR 1.821(d).

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 39-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 39-40 recite the phrase "modified pullulanase". It is not clear to the Examiner whether recitation "modified pullulanase" refers to the truncated pullulanase or whether other types of modifications of the pullulanase is included in the scope of the claim. Thus the scope of the above phrase is not clear to the Examiner.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 14-15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a truncated pullulanase produced by transforming a host cell with the polynucleotide comprising SEQ ID NO:1 encoding a truncated pullulanase, does not reasonably provide enablement for any polynucleotide isolated from any *Bacillus* that is at least 90% identical to SEQ ID NO:1. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the

prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 14-15 are so broad as to encompass any pullulanase encoded by a polynucleotide having 90% sequence identity to SEQ ID NO:1. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides (encoding said polypeptides), broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of a single pullulanase. It would require undue experimentation of the skilled artisan to make and use the claimed polypeptides. The specification is limited to teaching the use of SEQ ID NO: 1 as the polynucleotide encoding he truncated polypeptide but provides no guidance with regard to the making of variants and mutants or with regard to other uses. In view of the great breadth of the claim, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), the

claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any polynucleotide with 90% identity to the enzymes polynucleotide of SEQ ID NOS:1 because the specification does not establish: (A) regions of the protein structure which may be modified without affecting pullulanase activity; (B) the general tolerance of pullulanase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying amino acid residue with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including polynucleotides with an enormous number of amino acid modifications of the pullulanase with SEQ ID NOS:2 and 3. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA

1970)). Without sufficient guidance, determination of polynucleotides that are 90% identical to SEQ ID NO:1 and continue to encode a truncated pullulanase having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 5-7, 9-10, 14-15, 27-29, 31-40, 52-53, 55-61, 63-66 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deweer et al. (US 6,074, 854 filed 12-23-97, issued 6-13-2000) and McPherson et al. (Biochemical Soc. Trans., 1988, vol. 16(5) :723-724) or Albertson (Biochim. Biophys. Acta, Vol. 1354:35-39, 1997).

This rejection is based on printed publications and a patent. Claims 5-7, 9-10, 14-15, 27-29, 31-40, 52-53, 55-61, 63-66 in this instant application are drawn to a modified pullulanase from *B.deramificans* T89.117D with an amino acid sequence of SEQ ID NO:2, wherein the modification is a deletion of about 98, 100, 102, 200 amino acids from the amino terminus, wherein the modified pullulanase is produced by culturing a host cell comprising a nucleic acid which is at least 90% identical to SEQ ID NO:1 encoding a truncated pullulanase wherein the

host cell is *B.licheniformis* in which certain proteases are inactivated or eliminated. The claims are also drawn to compositions comprising the above modified pullulanase and compositions further comprising additional enzymes such as glucoamylase isolated from *Aspergillus* strains and wherein the modified pullulanase is 60 or 80% of the composition and wherein the composition is in the solid or liquid form.

Deweer et al. teach a pullulanase obtained from a Gram positive bacteria such as *B.deramificans* T89.117D produced by a method of culturing a host cell such as *B.licheniformis* in which certain protease genes have been inactivated. The reference also teaches the method of making the recombinant enzyme by obtaining the host cell transformed with a polynucleotide having more than 90% identity to SEQ ID NO:1 (see sequence alignment sent in the previous office action). The reference teaches the compositions either in the solid form or liquid form comprising pullulanase wherein it is of the order of 60% of the total enzyme concentration. The reference also teaches compositions comprising additional enzymes such as glucoamylase isolated from *Aspergillus* strains (see claims in the reference). However, the reference does not teach modification of pullulanase by way of deletion of about 100, 200 or 300 N-terminal amino acids.

McPherson et al. teach that pullulanases are significantly large enzymes when compared to other polysaccharide hydrolases and that this large size reduces the efficiency with which it can function by restricting access to internal alpha 1,6 bonds within highly branched substrates. The reference teaches that proteolytic digestion and computer-based sequence analyses are being used in the art to define a functional "core" pullulanase. The reference provides sources for such computer based homology searches. As an example the reference provides a schematic

illustration of the relative position of the 5 conserved "amylase" regions within a selection of hydrolases in comparison to the large *K.pneumoniae* pullulanase. The reference teaches that the long N-terminal region lacks any polysaccharide binding or catalyzing sites. McPherson et al. teach the modification of deleting nearly 170 amino acid residues from the amino terminal end which leads to approximately 30% higher activity than that of the native enzyme.

Albertson et al. also teach the modification of a pullulanase (from *C.saccharolyticus*), wherein nearly 381 nucleotides from the 5' region of the cDNA encoding a pullulanase was deleted resulting in a N-terminal truncated pullulanase. The reference also teaches that the deleted amino acid sequence is not essential for either activity or thermostability.

While both McPherson et al. and Albertson et al. do not teach a pullulanase isolated from a *Bacillus*, it appears that experiments involving truncation of N-terminal amino acids in pullulanase enzymes was well known in the art. These experiments appear to have been performed to determine the nature and the location of secretion signal, activity, catalytic site, transport across membrane and secretion into liquid medium.

It would have been obvious to one skilled in the art at the time the invention was made to combine the teachings of Deweer et al. with that of McPherson et al. and Albertson et al. to compare the large pullulanase provided by Deweer et al. with other *Bacillus* pullulanase just as taught by McPherson et al., followed by a method to make a modified pullulanase in which any number of amino acids up to at least a maximum of 381 amino acids from the N-terminal amino acids have been deleted. This is because Deweer et al. teach a pullulanase isolated from a Bacillus, *B.deramificans*, which is a very large size enzyme with more than 900 amino acids. McPherson et al. teach a method of increasing the efficiency of large size pullulanase by

determining and deleting non-essential amino acids in the N-terminal region. Albertson et al. and McPherson et al. demonstrate that deletion of up to at least 170 and 381 amino acids in such large size pullulanases does not affect the activity of the enzyme negatively but on the other hand increases the efficiency of the enzyme by nearly 30%. It would also be obvious for one skilled in the art to eliminate or inactivate protease genes in the expression hosts, such as Carlsberg protease or endo Glu C protease as Deweer et al. teach such inactivation of proteases such that the heterologous protein is not digested by the endogenous proteases.

Based on the above teachings, one of ordinary skill in the art would be motivated to delete N-terminal amino acids just as McPherson et al. by comparing and determining that N-terminal regions of large pullulanase do not have any conserved sequences for either activity or binding to polysaccharide and cleavage of such non-essential sequences results in higher efficiency of the enzyme. Those of ordinary skill in the art would also be motivated by Albertson et al. teaching in which up to 381 N-terminal amino acids have been deleted. One of ordinary skill in the art would have a reasonable expectation of success since Deweer et al. provide the nucleic acid encoding the pullulanase from *B.deramificans* in a host cell such as *B. licheniformis* in which protease genes have been inactivated and also provide the compositions comprising up to 60% of pullulanase in order to perform the modification.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

In response to the final rejection of the above claims previously, applicants have traversed the above rejection. Contrary to applicants argument, the reference of McPherson et al. is aimed at all pullulanases in general even though some of its focus is on the pullulanase of

Klebsiella. The reference clearly teaches that pullulanases in general are large enzymes and that truncation of pullulanases generally leads to an increase in the efficiency of the enzyme by 30%. That teaching by itself and also due to the well known fact in the art that pullulanases have industrial application would have motivated one of ordinary skill in the art to produce truncated pullulanase from large size pullulanases regardless of its source.

Albertson et al. also teach a similar aspect of pullulanases and even compare sequences from *Bacillus* and other bacterial species. Applicants argue at length that in addition to the conserved regions revealed by Albertson et al., they have disclosed two other conserved regions and those regions "Y" and "VWAP" are not taught by Albertson et al. Applicants also argue that they further disclose that the limits of amino acid truncations in the N-terminus of pullulanase would not go beyond the "Y" region and in response to the previous rejection, applicants have included the limitation of the truncated enzyme comprising the conserved "Y" region. Examiner has reconsidered such arguments and perused the references again and takes the following position. Applicant's argument that the there is a new "conserved" region not taught by the reference which has been identified only by them is weak. This is because, applicants have compared their sequence with only two other sequences (Figure 2) and while the "VWAP" sequence appears to be present in all three of the pullulanases the "Y" region does not. Furthermore, it is also not clear to the Examiner as to how applicants have concluded that the so called "Y" and "VWAP" regions are the conserved regions without comparing the instant pullulanase sequence with a significant number of other pullulanases drawn from a variety of microbial sources.

Examiner also takes the position that based on the teaching of McPherson et al. and Albertson et al. that deletion of up to at least 170 or 381 amino acids does not affect the activity of the enzyme negatively but on the other hand increases the efficiency of the enzyme by nearly 30%, it would have been obvious to those skilled in the art to delete any number of amino acids (20, 30, 40, 49, 98, 100, 101, 102 etc.) at least up to the first 200 amino acids in the pullulanase taught by Deweer et al. and such a deletion would still include the conserved regions that applicants have now included in the claims. Examiner has excluded claim 8 (and those depending from it) drawn to the truncated enzyme in which 300 amino acids have been deleted from the above rejection. According to applicant's own admission, the limit for deletion is up to amino acid Y at position 310. Therefore, the obvious deletion of first 200 amino acids would leave the amino acid at position 310 still attached to the enzyme which would now be 30% more active than the full length enzyme.

Therefore, for all the above reasons, Examiner continues to maintain the above rejection of claims under 35 U.S.C. 103(a) as being *prima facie* obvious.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Deweer et al. (US 6,074, 854 filed 12-23-97, issued 6-13-2000). This rejection is based upon the public availability of a patent publication. Claim 12 of the instant application is drawn to a modified pullulanase isolated from *B.deramificans*, wherein the modification is an addition of at least has at least one amino acid added to the amino terminus of a mature pullulanase amino acid sequence, wherein the added amino acid is alanine. Deweer et al. teach the modification of an identical mature pullulanase by at least one amino acid, i.e., addition, substitution, deletion of at

least one amino acid. However, the reference does not specifically teach that the added amino acid need to be alanine.

However, with the above pullulanase in hand followed by the teaching of modifying it by at least one amino acid, it would have been obvious to those skilled in the art to modify the enzyme of Deweer et al. by adding one amino acid anywhere in the sequence including the N-terminal and assay such modified enzymes of having the pullulanase activity. Since there are only twenty amino acids that can be used for modification, it would be obvious to those skilled in the art to use all or any of the twenty amino acids including alanine and select one or more of the modified enzyme that continues to have the activity. One of ordinary skill in the art would be motivated to do so in order to make a pullulanase that is simply different from that of an already patented enzyme in the art. One of ordinary skill in the art would have a reasonable expectation of success since there are only a limited number of amino acids that can be used for modification of an enzyme and Deweer et al. provide the mature pullulanase enzyme and also teach that a modification with at least one amino acid can be made along with techniques that can be used for making such modified enzyme.

Therefore, the above invention would have been *prima facie* obvious to those skilled in the art.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out

the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

Claims 8, 30, 54 are objected.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 6.30 a.m. to 3.00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned is 703-872-9306 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Manjunath N. Rao
April 30, 2004